1973

The Periapical Tissue Response to, and Sealing Ability of Apically Packed Dentinal Shavings

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THE PERIAPICAL TISSUE RESPONSE TO AND SEALING ABILITY OF APICALLY PACKED DENTINAL SHAVINGS

by

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

May, 1973

BIOGRAPHY

Robert J. Clayton was born in Streator, Illinois on December 23, 1943. He attended the local grade school system and was graduated from Streator Township High School in June of 1962. He entered the University of Illinois at Urbana, Illinois in the Fall of 1962 and majored in zoology.

In March of 1966, the author entered the University of Tennessee Dental School and was graduated with the degree of Doctor of Dental Surgery in March, 1969. For the next two years, he served as a dental officer with the United States Navy at Pensacola Naval Air Station, Pensacola, Florida.

He began his graduate studies in the Department of Oral Biology and clinical training in Endodontics under Dr. Franklin Weine at Loyola University in August of 1971.

Dr. Clayton is married to the former Christine Lanigan of Streator, Illinois and they have three children; Jeffrey, Cheryl, and Kelli.

DEDICATION

To my wife, Christine, whose love, understanding, and encouragement have been so very important during our many years.

ACKNOWLEDGEMENTS

To my Advisor, Dr. Hal McReynolds, whose constant guidance and constructive criticism have been of great value during this true learning experience, I wish to offer my sincere appreciation.

To Dr. Harold Gerstein and Dr. Joseph Gowgiel, whose advice and assistance have been most helpful, I wish to offer my thanks.

In addition, I wish to offer my sincere thanks to the Department of Endodontics for making possible the past two years.

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CHAPTER I

INTRODUCTION

In determining the rationale for endodontic therapy, the biology of the apical region must be understood. The apex must be viewed as a vital, dynamic tissue that is capable of growth, development and repair (39). Pulpal inflammation and infection may destroy the periapical cementum and periodontal ligament, but when the irritant has been removed, cementum may be deposited freely on teeth with previously vital or non-vital pulps (23)(25)(60).

This has led to the concept that complete removal of the affected pulpal tissue is a major prerequisite to periapical repair (24)(28)(54). Biologically, the cemento-dentinal junction would appear to be the only possible correct apical limit for this procedure (21)(39)(42). It will allow for a minimum inflammatory response and optimum healing potential (52).

Complete obliteration of the root canal space is also considered a prerequisite for periapical repair (24)(28)(54). Without a tight apical seal, secondary bacterial penetration of the filling material may occur. This rationale has led the dental profession in search of a nonirritating filling material that will produce a mechanical seal. Many methods have been

devised to test this mechanical seal (36)(44)(53).

Although filling materials are usually well tolerated by the periapical tissues, an ideal cementum bridge across the apical foramen, with attached periodontal fibers, is seldom obtained (8). Complete obliteration of the dentinal portion of the canal would allow for a biologic seal with cementum (11).

The root filling material assumes the role of an implant around which the connective tissue should close without reaction and without formation of a necrotic zone (30). Kuttler (31) believed that an ideal filling material should also stimulate the obliteration of the cemental portion of the canal with new cementum. Since contact of dentin with the periodontal tissues initiates cementum deposition, he suggested that dentin shavings implanted in the terminal portion of the canal might stimulate a more rapid periapical repair with new cementum. Some investigators have advocated apically packed dentin shavings which were altered in some fashion (15)(18)(19).

The purpose of this study is to investigate the periapical tissue response to, and the sealing ability of, apically packed dentinal shavings.

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Dentin Shavings and Root Canal Therapy

Several investigators have used dentin chips, prepared by different methods, as a root canal filling material. In 1939, Gottlieb (18) utilized a preparation containing dentin powder and Hilsitin salve. Dentin powder was prepared by placing extracted vital teeth in a 10% formalin solution. The dentin was removed and placed in a Petri dish which contained cotton saturated with formalin. A lentulo spiral was dipped into the salve and dentin powder. It was placed into the prepared canal and a gutta percha-cement filling was placed over the dentin-salve preparation. Histologic examination was used to demonstrate cementum repair of the apical opening in the dog five months after the treatment of a necrotic root canal.

In 1950, Gottlieb (19) offered further evidence that dentin powder was useful in root canal fillings. The dentin powder was prepared in the same manner, but it was now combined with a 10% sulfathiazole salve. Equal parts of the salve-dentin mixture were placed in canals followed by a gutta percha-sealer combination. Radiographs of human teeth filled in the above manner showed excellent results at the two-year recall. Additional

histologic sections of non-vital dog teeth demonstrated cementum repair over the dentin-salve mixture.

Kuttler (31) has suggested that the ideal filling material should stimulate the obliteration of the cemental portion of the root canal. According to the Kuttler technic, a rat-tail file is passed over the wall of a prepared, disinfected canal to gather dentin filings and to deposit them on a sterile glass slab. The master gutta percha cone is dipped into chloroform and inserted in dentin filings. The cone, with the adherent filings, is placed into the canal and accessory gutta percha cones with sealer are laterally condensed into the canal. Radiographic and clinical observations seem to indicate that dentinal shavings stimulate a more rapid periapical regeneration than in teeth of the same individual in which dentin filings are not used.

Erausquin (15) combined dentin chips with radicular pulp tissue to form an apical plug. He studied the periapical tissue response in rats to fifteen different types of root canal filling materials placed above the apical plug. They were evaluated according to inflammatory reaction, cementum response, and type of foramen closure. The initial response was favorable because there was no damage to the periapical tissues due to instrumentation. Furthermore, the tissues were not in contact with the irritating components of the filling materials. The long term

response was dependent upon how the filling material affected the pulpal remnants within the apical plug. The periapical tissue response depended upon the ability of the filling material components to penetrate the apical plug. Narrowing of the foramen was seen in many instances.

A number of investigators have mentioned dentin shavings which were inadvertently packed or pushed into the pulpal or periapical tissues. Seltzer et al (51) investigated the incidence of epithelial proliferation following various endodontic procedures in human subjects. A network of epithelial cells was found surrounding dentin filings that had been pushed beyond the apex during instrumentation. The same was true for other filling materials which extended into the periapical tissues. They recommended that all instrumentation and filling materials be confined within the root canal to decrease the incidence of epithelial proliferation, possibly with the eventual formation of a radicular cyst.

In a pulp capping experiment in 1964, Seltzer (49) observed that dentin chips, pushed into the pulp tissue during preparation, acted as a nidus for hard tissue formation. He also noted a predentin matrix had formed in apposition to the dentin chips within three days.

In another study, Davis (13) observed healing in dog root canals instrumented to within one millimeter of the radiographic

apex, but filled short. Several patterns of healing were described, but of particular interest was a healing pattern associated with apices that had been mistakenly instrumented to the radiographic apex and inadvertently packed with clean dentin shavings. Serial sections appeared to indicate that within four months the dentin filings had been completely covered by new cementum and the periapical tissues were free of inflammation.

In discussion of inadvertent packing of dentin chips into the apical constriction of the tooth, Moodnik (39) stated that dentin chips may aid in apical blockage and healing. This investigator concluded that it may be possible to establish a biologic apical seal with the use of an inoculum.

It appears that dentin fragments, used as an inoculum; i.e., implanted into various connective tissue sites; are capable of stimulating hard tissue formation. Linghorne and O'Connell (34) implanted autogenous dentin chips into surgically created osseous defects in dogs. Histologic examination demonstrated a significant increase in bone repair in the grafted defects and it appeared that the dentin chips were responsible for the osteogenic effect.

Urist and McLean (56) and Yeomans and Urist (63) have implanted decalcified dentin into several sites. A bone-induction system was the result. Yeomans and Urist (63) implanted decalcified dentin matrix, undecalcified dentin, and

decalcified bone in muscle, mandible, and tooth sockets of rabbits. Bone induction occurred within eight to twelve weeks in each host site when the implanted material was either decalcified dentin or bone matrix. Undecalcified dentin induced bone formation in 78% of the host sites, but only after a latent period of eight to twelve weeks.

The first intentional use of unaltered dentin shavings as a root canal filling material was briefly described by Gollmer (17) in 1937. He said, "The laws of replantation of human tissue should be observed, and these laws ordain that the material for repair should be taken from tissues of the tooth itself." Therefore, he prepared root canals with a special drill that would widen the canal when running in one direction and fill the canal with dentin shavings when running in the opposite direction. Histologic examination of dog teeth treated in this manner demonstrated that dentin shavings seemed to "unite" with cementum and that cementum was deposited over the apical foramen. After examination of Gollmer's photomicrographs, it appears that he packed dentin shavings into the myriad of accessory canals which make up the apical area of dog teeth. This gave the appearance of dentin "uniting" with cementum. Radiographic examination of human teeth showed no noticeable changes after eight to twelve months.

Later, Mayer (37) presented a technic in which root canal

preparation was short of the apical foramen in vital cases. The remaining apical pulp stump was covered with dentin chips and a paste filling material was placed in the remainder of the canal.

Ketterl (29) was the first to examine histologically the results of the Mayer technic. He treated human teeth and extracted them at intervals ranging from a few days to ten months. The filling material was always separated from the periapical tissues by a layer of dentin shavings. He stated that there were no signs of inflammation in the apical pulp stump if the filling material terminated one to two millimeters from the apex. After a period of ten months, cementum closure of the remaining root canal occurred in one specimen. The remaining ten-month specimens contained some cementum in the canal.

Mayer and Ketterl (38) later refined the original Mayer technic and presented it in detail. All cases were vital pulp extirpations and may possibly be thought of as partial pulpectomies. An engine driven reamer, with a square, flattened tip, was used to remove the pulp tissue one to two millimeters short of the apical foramen and to shape the canal. Dentin shavings were pushed ahead of the square tip of the instrument, thus acting as a dressing between the remaining vital tissues and the filling material. The canal was dried

and Diaket* was placed into the root canal preparation with a rotary instrument.

Over thirteen hundred teeth were treated in this manner and follow-up radiographs were obtained on 542 teeth. Eighteen months after treatment, 91% of the cases were noted as completely normal. In cases where there was underfilling or overfilling, good results were obtained 85% of the time.

In 1965, Ketterl (30) stated that serial sections of apical regions gave an objective criterion for success of root canal therapy. He evaluated Mayer's technic and reported spontaneous closure of the root canal, including lateral canals, in 95% of the teeth submitted for histologic examination. After an initial inflammatory reaction to vital pulp extirpation, the connective tissue became increasingly fibrous. Deposition of cementum narrowed the lumen of the root canal and resulted in closure in most cases. The best results were obtained when the fill was nine-tenths of a millimeter from the apex. This resulted in radiographic success 91% of the time and histologic success 73% of the time.

In a similar study, Waechter and Pritz (58) also demonstrated hard tissue formation at the apex following root canal therapy in twenty human teeth. They placed dentin chips over

*Premier Dental Products Co., Philadelphia

the vital pulp stump and filled the remainder of the canal with Diaket. Two to five years after treatment, the teeth were prepared for histologic examination. An "osteocementum" substance formed at the apex and appeared to seal the canal. This occurred only when dentin chips formed the wound dressing. The osteocementum "seal" did not fill the entire canal below the dentin chips, but only at the wound surface within the pulp canal. Healthy periodontal tissue was usually found within the apical portion of the canal.

Waechter and Stockinger (57) treated twenty human teeth in the same manner and obtained variable results after intervals of 5 months to 6 years. There was evidence of some hard tissue formation, but the results were not consistent.

Baume et al (3) were the most recent group to investigate the use of dentin filings over vital partial pulpectomies on thirty human teeth. Smooth-ended files were used to remove the pulp tissue at a distance three to five millimeters from the apex. A file was rotated alternately clockwise and then counterclockwise, thus creating an immediate dentin cap on the remaining vital pulp stump. Zinc oxide and eugenol was used to fill the remainder of the canal and chamber. The teeth were prepared for histologic examination and evaluated according to the following criteria: degenerative connective tissue changes, osteodentin formation, and inflammatory responses. The extent

of osteodentin formation seemed to depend on the thickness of the dentin chip layer and the level of pulpotomy. More osteodentin was formed when the dentin plug was thicker and when the amputation site was nearer the foramen. Serial sections revealed that the osteodentin layer did not form a perfect seal.

From these investigations, it appears that dentin chips or fragments can induce hard tissue formation in connective tissues. It has also been shown that dentin chips placed over apical pulp tissue give a minimal inflammatory response and induce formation of an osteocementum substance. This material tends to constrict the apical foramen and may possibly be able to form a biologic seal of the apical foramen. This study will attempt to investigate the response of the periodontal ligament to unaltered dentin chips condensed apically following root canal preparation.

B. Methylene Blue in Endodontic Research

Methylene blue; also known as methylene blue chloride, Swiss blue, and tetramethyl-thionin chloride; is a basic dye of the quinoneimine group, thiazin subgroup (4). It was first prepared by Caro in 1876 from dimethylaniline and thiosulfuric acid. The dye has a molecular weight of 319. Methylene blue is generally used by histologists as a nuclear stain (22). Much of its value as a nuclear stain is due to metachromasia

and its oxidized products, the azures (33). It is used by bacteriologists in the analysis of milk. The dye is also employed as a non-toxic vital stain for nerve tissue due to its tendency to form the colorless, leucomethylene blue, when tissue is deprived of oxygen. Methylene blue is also used in combination with other dye formulations. It is an ingredient in stains such as Wrights, Giemsa, Leishman, and Jenner.

According to Barbosa and Peters (4), methylene blue was one of the first dyes used as a vital stain. The biochemical behavior of methylene blue at the cellular level has only been recently investigated. Barbosa and Peters (4) reported that methylene blue forms an ATP-dye particle complex, thus reducing available cellular ATP. It also has an inhibitory effect on acetycholinesterase. Nuclear staining by methylene blue may be due to the phosphate groups of the nucleic acids (45). Methylene blue has also been used in a qualitative procedure for the determination of mucopolysaccharides.

The behavior of methylene blue in dentin was described by Bodecker and Lefkowitz (9) in 1946. They noticed a marked difference in the distribution of dye particles in vital and non-vital teeth. In vital teeth, the dye distributed or transported mainly within the dentinal tubules. In non-vital teeth, the dye diffuses in all directions, not only along the dentinal tubules, but also between the tubules.

In 1962 Anderson and Ronning (1) described the diffusion of dye from an exposed dentin surface inward and from the pulp toward the enamel surface. Cutting the dentin surface reduced the permeability of the tubules. Living processes are not completely responsible for penetration of the dye into dentin since dye penetrates dentin tubules of extracted teeth and irregular dentin (64). Diffusion, capillary attraction, or differences in partial pressures may explain the penetrability of the dye.

Dyes, methylene blue in particular, have been used for many years to demonstrate the sealing ability of endodontic filling materials. Webster (61) was the first to investigate root canal fillings with a dye. Glass cylinders were filled with cotton, gutta percha, zinc phosphate, and chloropercha. One end of each cylinder was dipped into a red dye for 24-72 hours. All materials demonstrated marginal to complete penetration by the dye but chloropercha produced a good seal 70% of the time.

Schroeder (46) was the next to use a dye, methylene blue, to test a root canal filling material. He tested a paste filling and found no dye penetration when the test system, dye placed intradentally, was centrifuged.

In 1958 Stewart (53) compared the sealing ability of three root canal sealers. He prepared root canals and filled them with silver points and gutta percha, utilizing lateral condensa-

tion. Some teeth were placed in methylene blue dye baths and permitted to remain in an incubator at 37° for up to six months. Other teeth had dye placed within the canals, above the sealer, prior to incubation. They were removed, dried, and sectioned horizontally. The degree of marginal penetration was similar, regardless of dye placement. There was no evidence of complete penetration through the root canal filling materials studied.

Sekine, et al (48) tested zinc oxide eugenol, copper cement, and gutta percha. The materials were placed in glass tubes and dipped in methylene blue for 10 minutes to seven days. Twenty-four to forty-eight hours gave optimum penetration of the materials by the dye.

Curson and Kirk (12) assessed root canal sealing agents by placing them in glass tubing and then immersing them in methylene blue for one to thirty days. Zinc oxide eugenol sealers produced a good seal, but increased immersion time gave some marginal leakage.

Antoniazzi et al (2) tested the sealing ability of root canal sealers alone and in combination with gutta percha. They filled extracted teeth and placed them in methylene blue, both immediately and after the sealers had set. They found no significant difference in marginal penetration if gutta percha was used in combination with a sealer. These investigators stressed the importance of testing materials in vivo.

Blair (7) evaluated sealing properties of endodontic filling materials in extracted human teeth. He tested gutta percha and silver points with three sealers. The root canals were filled and the teeth immersed in a 0.25% methylene blue dye solution and stored at 37° for 24 hours. The teeth were sectioned longitudinally so that the entire intraradicular surface could be examined. Solid core materials with sealer showed some marginal penetration, but there were no significant differences in the materials used.

Scoralle (47) attempted to test the sealing ability of root canal filling materials in vivo. He tested different types of root canal fillings, utilizing several different technics, with 1% methylene blue dye placed intradentally. The dye remained in place for 48 hours and the teeth were then extracted, sectioned longitudinally, and examined for marginal penetration of the filling material by the dye. It was found that the dye was capable of penetrating all filling materials to some degree.

Methylene blue was chosen to test the sealing ability of a gutta percha-sealer combination and dentin chips condensed into the root canal. It has been used for many years to test the marginal penetration of fillings. The dye molecule is smaller than a bacterium. It is relatively easy to evaluate the results of canal sealing ability with methylene blue and it

gives comparable, if not identical results, to other methods of evaluation (16)(44).

Methylene blue is lost during preparation of thin sections for histologic examination since it is extracted during dehydration (33). Preliminary work with the dye and 7 micron sections verified this observation, but the dye was found to be readily visible in the mounted blocks.

The sealing ability of root canal fillings will be evaluated by observing dye penetration in the mounted blocks as longitudinal sections are being prepared for histologic examination.

CHAPTER III

MATERIALS AND METHODS

The periapical tissue response to and the sealing ability of apically packed dentinal shavings were demonstrated in the following manner. Four mongrel dogs, ranging in weight from 19.5 kilograms to 23.0 kilograms, were utilized for this experiment. They were treated and housed at the Loyola University Medical Center Animal Research Facility. The same treatment regimen was adhered to for each animal involved.

Each animal was anesthetized with an intravenous injection of pentobarbital sodium*. The initial dose was 30 milligrams per kilogram of body weight (26). Supplemental amounts were administered to maintain adequate anesthesia. An intramuscular injection of atropine sulfate** (0.4 milligrams per cc) was administered to decrease oral secretions (35). The animal was secured to the operating table and a spring-retained mouth prop was placed in position over the cuspid teeth. Pre-treatment radiographs were taken and developed.

The premolar teeth to be treated were swabbed with 70% alcohol and pieces of 4x4 cotton gauze were placed in the buccal

*Holmes Serum Co., Inc., Springfield, Ill.
**Chicago Veterinary Supply Co., Chicago, Ill.

vestibule and under the tongue. The gauze packs, jaw retractor, and atropine injection all served to isolate the teeth to be treated. An access cavity preparation was made by cutting the occlusal surfaces of the teeth flat with a crosscut fissure bur until the pulp horns were observed. A round bur was used to extend the preparation to include the entire pulp chamber and to prepare the access cavity for an amalgam seal.

The canal contents were broached and a #15 file* was placed in each canal. A radiograph was taken to establish the canal length. The canals were enlarged using standardized endodontic instruments until clean, white dentinal shavings were obtained along the entire instrument working length. Copious amounts of physiologic saline were used to flush debris from the canal. Another radiograph was taken at this point and the instrument size and length were recorded (Charts I-IV).

An apical perforation length was determined from the radiograph and the known instrument length. The perforation length was recorded (Charts I-IV). A #20 engine driven reamer was used to perforate the remaining tooth structure to the radiographic apex. Polyethylene tubes were used to make a firm, positive stop on the power driven reamer. This allowed perforation to, but not past, the radiographic apex. One

*AAE Standardized Instruments

prepared canal in each animal was not perforated. This served as a control.

The prepared root canals were iriigated and completely dried with absorbant paper points. At this point, the canals were placed into one of four groups.

Group I served as the control for this experiment. The non-perforated preparations and the perforated preparations in Group I did not contain a root canal filling. Employing a 1cc tuberculin syringe, a 1% methylene blue dye solution was placed within the canal of each tooth in Group I. A small piece of gutta percha was placed over the dye and an amalgam filling was placed in the occlusal access preparation.

Group II preparations were filled with gutta percha* and Wach's Paste**. A lateral condensation filling technique (62) was employed. A dye reservoir was made within the canal by using a warm plugger to remove all but three millimeters of gutta percha at the apex. Methylene blue dye solution (1%) was placed within the root canal and gutta percha and an amalgam seal were placed in the same manner as the first group.

Group III preparations were filled in the following manner. A file, one size larger than the final instrument used in the preparation of the canal, was used with a downward rasping or

*Mynol Chemical Co., Broomall, Pa.
**King's Specialty Co., Fort Wayne, Ind.

filing action to pack dentinal shavings apically. After several downward strokes, a #20 file and the final working instrument were used to condense the shavings. These procedures were continued until a two millimeter layer of dentin shavings had been condensed into the apical area of the preparation. Methylene blue dye solution (1%) was placed into the canals and they were sealed as in the previous two groups.

Group IV specimens were filled with dentin shavings and gutta percha plus sealer. The dentin shavings were packed into the apical two millimeters as in Group III and gutta percha plus sealer was laterally condensed as in Group II. A dye reservoir was prepared by removing all but three millimeters of gutta percha. Methylene blue dye (1%) was placed into the reservoir and the preparations were sealed. A final post-treatment radiograph was taken.

Following the preparation, filling and sealing of the premolar teeth, the dogs were returned to their housing area. They were placed on a diet of soft dog chow and water until the experiments were terminated.

The dogs were sacrificed at intervals of one (Dog A), two (Dog B), three (Dog C), and four (Dog D) months. An intravenous injection (8-10cc) of Totaltox* was administered to each

*Chicago Veterinary Supply Co., Chicago, Ill.

animal. The mandible was removed and sectioned, anterior to the first premolar and posterior to the first molar, with a large bone saw. A model trimmer was used to remove the lower border of the mandible. Each block was placed in a 10% neutral formalin solution for a period of 10 days and rinsed in running water for 24 hours.

A sodium citrate-formic acid solution (40) was used to decalcify the specimens. During decalcification, the specimens were further trimmed to obtain a block for proper embedding size. Decalcification was completed in forty to sixty days and a thorough (24-hour) water rinse followed. The trimmed blocks were dehydrated in ethanol, embedded in paraffin, and sectioned at 7 microns. Serial sections of the apical foramen area were mounted on glass slides. The sections were deparaffinized, hydrated and stained with hemotoxylin and eosin (33).

As the sectioning procedure reached the root canal space, the methylene blue dye was readily visible in the mounted block. Penetration of the dye into and along the side of the filling materials was recorded (Charts V-IX) and representative photographs of the mounted blocks were taken.

Stained histologic sections were examined using a light microscope. The sections were evaluated according to the amount and type of inflammation present in the periapical tissues; the amount of cementum, dentin, and bone apposition

or resorption; and the organization of connective tissue.

The radiographs were evaluated in the following manner. Post-treatment radiographs were compared to radiographs taken at the time of sacrifice. They were projected and three examiners graded the periapical tissue response as to the continuity of the lamina dura, the width of the periodontal ligament, and the size of any periapical radiolucency. The results were recorded in Tables V-IX.

CHAPTER IV

RESULTS

A. General

The animals were examined at the termination of the experiment. Each one appeared healthy and demonstrated a slight gain in weight. The teeth were examined for defects in the amalgam seal and for possible fractures of the teeth. The soft tissues were studied for the development of draining sinus tracts. Dog B had fractured several crowns which resulted in lost amalgams and contaminated root canals. No sinus tracts were present in any of the animals. Radiographic observations were made at the termination of the experiment and compared to post-treatment radiographs. The radiographic changes, which ranged from no periapical change to the development of large, diffuse periapical radiolucencies, are listed in Tables V-IX. A radiographic pattern for each group could not be distinguished but radiographic changes were more pronounced in the more posterior premolars. In general, a correlation between the radiographic and histologic pictures could not be made.

B. Dye Penetration

The sealing ability of two types of root canal filling

materials was evaluated by placing methylene blue intradentally and examining the specimens at one, two, three, and four month intervals. The findings were very consistent and will be reported in groups, irrespective of the examination period. Individual canal radiographic and dye penetration results are available in Tables V-IX.

Preliminary work indicated that dye was readily visible in the mounted block after the specimen had been decalcified, washed in water, dehydrated, embedded in paraffin, and sectioned. Dye had penetrated into dentin tubules adjacent to the dye reservoir in canals filled with gutta percha and sealer, but no marginal penetration was noted. One could also see dye penetration into the dentin chip layer and diffusion of the dye into the dentin tubules above the dentin chip layer. There was no penetration of the dye into tubules below the surface of the dentin chips, nor was there ever complete penetration of the dentin chip layer by the dye (Figure 1).

The sealing ability of the gutta percha-sealer combination was tested in Groups II and IV. The dye was visible in all specimens above the apical segment of the gutta percha-sealer fill. There was no apparent marginal penetration of the filling material by the dye and the dye was not visible in the dentin tubules (Figure 2).

Group III root canals showed partial penetration of the

dentin chip layer by the dye. The dye did not appear to completely penetrate the dentin chip layer nor was the dye present in the dentin tubules (Figure 3). There was no trace of dye in one canal. Group I consisted of control preparations. Dye was not visible in the canals which had apical perforations. Dye was visible in the apical third of most non-perforated controls (Figure 4). There was no dye present in the dentin tubules in any of the control specimens.

C. Histology

A relatively consistent histologic pattern developed in the periapical tissues in response to endodontic procedures and to the filling materials tested. The results of each group will be presented in the four to sixteen-week specimens, and exceptions to the general patterns will be noted.

1. Group I Specimens

The instrumentation procedure itself produced a pattern of periapical response. Simple cleansing and shaping of the root canal (Group I, non-perforated) initially produced a very small resorptive defect in the bone with mild chronic inflammation in the accessory canals and periodontal ligament. After eight weeks a few scattered, chronic inflammatory cells were present in the periodontal ligament. A cementoid substance had been deposited in the accessory canals. A complete lamina dura and

intact, well organized periodontal ligament were present twelve weeks after the procedure (Figure 5).

In addition to cleansing and shaping the root canals, it was necessary to perforate the apex of those which were to be filled (Group I, perforated). The perforation procedures initially produced a severe chronic inflammatory reaction with resorption of cementum, dentin, and bone. A relatively large granuloma had developed with necrotic cells present in the unfilled root canal. By twelve weeks, the granuloma had greatly reduced in size, but one could still see evidence of the resorptive defect (Figure 6). A mild chronic inflammatory infiltrate still persisted in the periodontal ligament and surrounding connective tissue spaces. The accessory canals appeared to be vital.

2. Group II Specimens

Each canal in Group II (Gutta percha-sealer) had a small amount of sealer extruded into the periodontal ligament. At four weeks, a large resorptive defect had been produced at the expense of the bony lamina dura. Very little, if any, cementum resorption had occurred. One could see particles of sealer in the periapical tissues surrounded by an eosinophilic staining material, liquifaction necrosis. There were multiple chronic inflammatory cells in a zone adjacent to this material. There was active resorption of the bone in contact with the loose

connective tissue-chronic cell zone and, apparently, active apposition of bone on the reverse side of the spicules. At subsequent time intervals, the connective tissue became increasingly fibrous in nature and tended to wall the material off from the surrounding tissues (Figures 7 and 8).

3. Group III Specimens

Group III specimens consisted of dentin chips condensed into the apical preparations of the first and second premolars. Very small resorptive defects were observed in the lamina dura of four-week specimens. Connective tissue from the periodontal ligament had proliferated into the root canal space and had contacted the condensed dentin chips (Figures 9-12). There was an appearance of granulation tissue in some cases, but in others the connective tissue was similar to a well-organized periodontal ligament-like structure. Osteocementum had formed adjacent to the dentin chips. A very mild chronic inflammatory cell infiltrate was present in the widened periodontal ligament space.

In eight-week specimens one could see the resorptive defect, but there were definite signs of repair in the cementum. Connective tissue in contact with the dentin chips had laid down increasing amounts of osteocementum (Figures 13 and 14). Accessory canals appeared to contain vital tissue, and deposition of cementoid resulted in narrowing of some canals. The twelve and sixteen-week specimens demonstrated additional

osteocementum formation against the dentin chip layer and cementoid formation circumferentially within the main canal. The connective tissue, within the canal, was well organized and appeared to resemble periodontal ligament. Apical cementum also showed repair (Figures 15-17).

4. Group IV Specimens

Root canal fillings in Group Iv contained a gutta percha-sealer combination above the dentin chip layer, which was in contact with the periapical tissue. All Group IV fillings were performed on fourth premolars. The first specimens observed (four weeks) had large granulomas and actively resorbing defects which equally affected the bone and cementum. A moderate chronic inflammatory infiltrate extended along the periodontal ligament for some distance. Some accessory canals contained inflamed tissue. In the two-month dogs, one could still observe the extent of the resorptive defect, but there appeared to be evidence of some cementum repair. Connective tissue had proliferated into the canals and was in contact with the dentin chips. As in Group III specimens, osteocementum had been deposited adjacent to the dentin chips. Inclusion of cells in this material gave it the appearance of cellular cementum. Accessory canals appeared to contain vital tissue.

Twelve-week specimens still presented resorptive scars in bone and cementum, but there was excellent repair by osteocemen-

tum against the dentin chips and cementoid circumferentially along the canal wall. There was evidence of bone apposition with granulation tissue occupying the widened periodontal ligament space (Figure 18).

5. Exceptions to the General Patterns

In Group III, two twelve-week specimens had chronic inflammatory cells packed against the dentin chips. The resorptive defect contained granulation tissue in contact with these cells. Accessory canals contained necrotic tissue which contributed to a chronic inflammatory infiltrate in the widened periodontal ligament space.

In one canal of Group III the perforation length was too long and a defect was present in the bone below the tooth apex. The defect was nearly the same shape as that of the engine-driven reamer. Periodontal ligament repair was excellent, but the bony defect was the center of a small granuloma. Dentin filings had been forced into the periapical tissues by the reamer and they appeared to be centers of hard tissue formation (Figure 19).

Dog B fractured several amalgams. Group I had one canal containing no filling. An abscess developed with extensive resorption of bone, cementum, and dentin. Other amalgams were lost in Dog B, but repair patterns did not appear to deviate from those of the specific groups involved.

Some sixteen-week specimens added further support to the pattern of Group III repair. Increased amounts of osteocementum and cementoid-like material had formed within the canals and periodontal ligament organization was excellent. However, the remainder of the treated root canals demonstrated an extensive inflammatory response with active resorption of all supporting structures. The inflammatory response extended into adjacent marrow spaces and necrotic bone was frequently observed.

CHAPTER V

DISCUSSION

A. General Procedures

Hermetic sealing of the canal has been considered an important phase of root canal therapy for many years, although the exact location of the hermetic seal has been a controversial subject. Grove (21) and Orban (42) claimed that the cemento-dentinal junction is the most logical position for this seal. Kuttler (31) suggested filling to the cemento-dentinal junction with a material that would induce cementum formation over the apex. Such thoughts and philosophies were the impetus for this investigation.

The relatively small numbers of root canals treated in this experiment may limit the scope of the conclusions. It is also important to consider that the dog, the experimental animal in this investigation, is estimated to have a more rapid healing rate in its periodontium than that of the human; approximately 2.5 times faster (10). On the other hand, there is also evidence that dog periapical tissues are more sensitive to root canal therapy than those of the human. Presumably, this is due to the vast number of accessory canals branching from the main canal at the cemento-dentinal junction (5)(14)(19)(43).

This junction acts as a natural "stop" in the dog root canal system and preparation of the dentinal portion of the canal presents no real problem. In order to study periapical response, one must perforate the final cemental portion of the canal. Perforation with a small instrument would allow the filling material placed in the canal to contact the connective tissues. This provides a periapical system similar in morphology to, but more sensitive in response than, human periapices.

In this study, root canals were filled in a particular pattern in order to facilitate placement of dye into a reservoir of suitable size. Groups I and III needed minimal room for filling materials and, therefore, they were placed in the shorter first and second premolars. Group IV required more room for filling materials and was always placed in the longest root available, the fourth premolar. This arrangement provided two distinct test areas for dentin chips and the possibility of observing variations in periapical response within the same animal. Group I provided a control for instrumentation procedures and Group II provided a known or predicted response to an accepted root canal filling technic (gutta percha-sealer) (14).

B. Dye Penetration

Since it is considered important to obtain a periapical

seal during endodontic therapy, an attempt was made to evaluate the sealing ability of a dentin chip filling. Erausquin (15) is the only worker who has alluded to the sealing ability of dentin chips. He filled root canals with a dentin chip-pulpal remnant plug and noted that medicaments tended to diffuse into the periapical tissues. This may well have been due to the presence of the pulpal remnants within the "solid core" of dentin chips.

In the present investigation, an in vivo pilot experiment produced a dye penetration pattern in dentin similar to those found by other investigators; namely, penetration of dye into the dentinal tubules above the level of the filling material, and marginal penetration of the filling material itself (7) (20)(53). Scoralle (47) has also observed typical dye penetration patterns in a forty-eight hour in vivo penetration study. The same pattern was observed in a pilot specimen (dye present intradentally for twenty-four hours) that had been processed for histologic sectioning.

Results of the present in vivo study, in which dye was present for extended periods of time, were quite different. This may have been due to extraction of the dye during preparation of the specimen for sectioning (33), or it could have been due to an exchange of materials within the dentinal tubules in the in vivo system. Wasserman (59) demonstrated that exchange

of phosphate ions still continues in the dentinal tubules in spite of pulp tissue removal. Dye was present in the apical segment of the non-perforated group, but the presence of dentin chips in accessory canals of this group may have been responsible for this condition.

Determining the presence and amount of dye penetration at extended periods of time may not be a reliable in vivo test system. Isotope penetration studies over extended periods of time may present the same difficulties. However, if an exchange of materials does occur, a systems analysis, utilizing a radioactively labeled dye particle, may reveal the location of the end products.

Sealing ability of root canal filling materials should also be tested in vivo by a bacterial penetration method. During this investigation, Dog B presented this type of system when five amalgams were fractured and lost, thus exposing the filling materials. It was not possible to determine how long the teeth had been exposed to oral contamination, but a periapical radiolucency had developed in one Group I specimen. Most investigators agree that it takes two to three months to develop periapical radiolucencies (5)(6). If all fractures did take place at the same time, then there was an adequate test of the sealing ability of the different filling materials. The responses of the other groups indicated that their canals had

been properly sealed.

The poor response of the mandibular specimens in Dog D may have been due to the absence of dye in those canals of this animal. The bacteriocidal effect of the dye may have controlled any contamination of the canals during preparation and filling procedures (4). This is unlikely, however, because the dye was present in maxillary teeth of Dog D and the same negative response was observed.

C. Histology

In all cases, root canal preparation led to a resorptive defect in the lamina dura and cementum. This finding is consistent with those of other investigators (6)(14)(32)(50). In addition, perforation of the apex appeared to increase the inflammatory response and somewhat delayed healing. Marked histologic differences were observed in resorptive defects between the more anterior premolars and the fourth premolar. Similar findings have been observed radiographically by Bhaskar (6). It is unlikely that the difference in response was due to an uncontrolled perforation technic, since the more anterior premolars were perforated exactly to the periodontal ligament and the fourth premolar was perforated beyond the periodontal ligament. The difference in response seems to be a result of the variance in the proximity of the cemento-

dentinal junction to the apex (a larger amount of inflamed tissue remaining in the accessory canals in the more posterior premolars). In relation to this, Davis (3) has noted increased periapical inflammation when necrotic tissue remained in accessory canals.

Root canals in Groups III and IV were filled to the cementodentinal junction; thus, a variation existed in the distance of the dentin chips from the periodontal ligament. In effect, "hollow tubes" of differing lengths existed in most cases. Torneck (55) claimed that the "hollow tube" must be of sufficient diameter to allow tissue "bridging." Furthermore, Davis (13) has demonstrated that an 0.8mm diameter perforation produces excellent experimental results, but this large size is not clinically feasible. In this study, a 0.2mm diameter perforation produced an excellent result and, in addition, it has clinical application.

Unlike many investigators who studied the responses of radicular pulp tissue to dentin chips, this study has investigated the response of periapical connective tissues to dentin chips. Notwithstanding the fact that the entire procedure of radicular pulpotomy is dependent upon an accurate diagnosis of an uninflamed, intact radicular pulp, present dental methods do not allow making this diagnosis.

Formation of osteoid-like material within the root canal,

after total pulp removal, confirms the findings of Nygaard-Ostby (41) and Davis (13). Furthermore, this study has demonstrated that osteocementum formation occurred in apposition to the layer of packed dentin chips. The apical perforation appeared to be completely obliterated in some specimens with this material. Nevertheless, since every section could not be accounted for, this investigator does not claim that complete closure had taken place.

Inadvertently, one specimen was perforated two to three millimeters beyond the apex. This created two defects; one within the periodontal ligament and tooth apex and another in the lamina dura. In this case, the root canal foramen contained a dentin chip layer or inoculum, and excellent repair had taken place within four weeks. On the other hand, the bony defect did not contain a dentin inoculum and a granuloma persisted. Similarly, Yeomans and Urist (63) have demonstrated this phenomenon in bony defects of rabbit mandibles.

Two of the canals containing dentin chips exhibited persistent periapical inflammation. However, this may have been due to inadequate canal instrumentation. In one premolar root, two canals were present and this was not initially recognized. Vital pulpal remnants may have been condensed, along with dentin chips, into the distal canals of both teeth. Erqusquin (15) has noted periapical breakdown in this situation.

The gutta percha-sealer specimens developed a type of response consistent with those described by other investigators (6)(14)(27). Although resorptive defects did develop, they were usually at the expense of the bone. The sealer caused necrosis of cells immediately adjacent to the cementum, thus inhibiting resorption of cementum. Nevertheless, cementum resorption is known to occur at later time intervals (5)(6). A significant difference was that, unlike control specimens or the dentin chip fillings, no hard tissue formation took place when sealer was extruded.

Some canals in Dog D demonstrated repair and followed group response patterns. However, the majority of the specimens presented uniform breakdown of all supporting structures, with infiltration of chronic inflammatory cells into all connective tissue spaces. This seemed to be consistent with the idea that gross contamination of root canals, as opposed to latent breakdown of any particular filling material, was responsible for the pathologic condition of most of the periodontium in Dog D.

This study has raised several questions and has presented ideas for future investigations. (1) Would methylene blue be visible in the dentinal tubules after long intradental periods if the teeth were not processed for histologic examination? (2) Would the same results be obtained if dentin chips were

employed in long-term studies? (3) Would the results be different if there was a change in the diameter of the apical perforation? (4) Does the thickness of the dentin chip layer affect the results? (5) Can these results be extrapolated to the human system?

Further investigation of these questions may facilitate an understanding of the capability of periapical tissue to respond to root canal manipulation and filling materials.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The periapical tissue response to and the sealing ability of apically packed dentin shavings was studied. Sixty-four root canals in four mongrel dogs were prepared to their radiographic apices and filled with (1) a gutta percha-sealer combination, (2) dentin chips condensed apically, and (3) dentin chips backed by a gutta percha-sealer combination. A fourth group contained no filling material. Methylene blue dye was placed intradentally prior to the placement of an amalgam coronal seal and the experiment was terminated at one, two, three, and four-month intervals. The specimens were prepared for histologic evaluation and dye penetration of the filling materials was recorded from the mounted block. The results of this study lead to the following conclusions:

(1) Dentin chips enhance the formation of osteocementum in the periapical region;

(2) Excess sealer appears to inhibit hard tissue formation in short-term experiments;

(3) Two millimeters of dentin chips appears to provide an adequate seal against methylene blue dye penetration *in vivo*;

(4) A gutta percha-sealer combination, utilizing lateral condensation, appears to seal against methylene blue dye penetration *in vivo*;

(5) Larger resorptive defects occur in the more posterior premolars;

(6) Periapical connective tissue can proliferate into a 0.2mm diameter apical opening with subsequent osteocementum formation.

CHAPTER VII

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CHAPTER VIII

APPENDIX

A. TABLES - B. FIGURES

Symbols used in TABLES I thru XVIII:

R = right
L = left
M = mesial
D = distal
P = perforated
NP = not perforated
L = mandibular
U = maxillary
* = canal length (mm)
# = instrument size

TABLE I

CANAL PREPARATION - Dog A

Premolar Canal	Group	Cleanse & Shape	Perforation Length (mm)	Filling Material
L1	III	* 8.0-#50	9.0	Dentin
L2M	I	9.0- 50	11.5	Control (P)
L2D	III	10.0- 50	11.5	Dentin
L3M	II	10.5- 50	12.0	Gutta percha
L3D	II	10.5- 50	12.0	Gutta percha
L4M	IV	12.5- 60	14.0	Dentin - Gutta percha
L4D	IV	12.5- 60	14.0	Dentin - Gutta percha
R1	I	8.0- 45	NP	Control (NP)
R2M	III	10.0- 50	11.0	Dentin
R2D	III	9.5- 50	10.5	Dentin
R3M	II	11.0- 50	12.0	Gutta percha
R3D	II	10.5- 50	11.5	Gutta percha
R4M	IV	13.0- 60	14.0	Dentin - Gutta percha
R4D	IV	12.5- 60	13.5	Dentin - Gutta percha

NOTE:

(1) Mandibular premolars were treated.

(2) Treated canals contained dye solution.

TABLE II

CANAL PREPARATION - Dog B

Premolar Canal	Group	Cleanse & Shape	Perforation Length (mm)	Filling Material
L1	III	* 9.5-#50	10.5	Dentin - contaminated
L2M	I	11.0- 50	NP	Control (NP)
L2D	III	11.0- 60	12.0	Dentin
L3M	II	13.0- 60	14.0	Gutta percha
L3D	II	12.0- 55	13.0	Gutta percha
L4M	IV	14.0- 60	16.0	Dentin - Gutta percha
L4D	IV	13.5- 60	15.0	Dentin - Gutta percha
R1	III	9.5- 50	10.5	Dentin - contaminated
R2M	I	11.5- 40	12.5	Control (P)
R2D	III	11.5- 50	12.5	Dentin
R3M	II	13.0- 60	14.0	Gutta percha
R3D	II	12.0- 60	13.0	Gutta percha
R4M	IV	15.0- 60	16.0	Dentin - Gutta percha
R4D	IV	14.0- 60	15.0	Dentin - Gutta percha

NOTE:

(1) Mandibular premolars were treated.

(2) Treated canals contained dye solution.

TABLE III

CANAL PREPARATION - Dog C

Premolar Canal	Group	Cleanse & Shape	Perforation Length (mm)	Filling Material
L1	I	*10.0-#60	NP	Control (NP)
L2M	III	12.0- 60	13.0	Dentin
L2D	III	12.0- 60	13.0	Dentin
L3M	II	13.0- 60	14.0	Gutta percha
L3D	II	12.5- 80	14.0	Gutta percha
L4M	IV	15.0- 60	16.5	Dentin - Gutta percha
L4D	IV	14.0- 80	16.0	Dentin - Gutta percha
R1	I	10.0- 50	11.0	Control (P)
R2M	III	12.0- 55	13.5	Dentin
R2D	III	12.0- 55	14.0	Dentin
R3M	II	13.0- 70	15.0	Gutta percha
R3D	II	12.0- 80	14.0	Gutta percha
R4M	IV	15.0- 80	17.0	Dentin - Gutta percha
R4D	IV	15.0- 80	16.5	Dentin - Gutta percha

NOTE:

(1) Mandibular canals were treated.

(2) Treated canals contained dye solution.

TABLE IV

CANAL PREPARATION - Dog D

Premolar Canal	Group	Cleanse & Shape	Perforation Length (mm)	Filling Material
LL1	III	*10.0-#55	11.0	Dentin
LL2M	I	12.0- 60	13.0-60	Control (P)
LL2D	I	12.0- 60	NP	Control (NP)
LL3M	III	14.0- 80	14.5	Dentin
LL3D	II	13.0- 80	13.5	Gutta percha
LL4M	IV	15.0- 80	16.5	Dentin - Gutta percha
LL4D	IV	15.0- 80	16.5	Dentin - Gutta percha
UL2M	II	12.5- 80	13.5	Gutta percha
UL2D	III	11.5- 80	12.5	Dentin
UL3M	IV	12.5- 80	13.5	Dentin - Gutta percha
UL3D	I	12.5- 80	NP	Control (NP)
LR1	II	10.5- 55	11.0-55	Gutta percha (overfill)
LR2M	III	12.5- 80	13.5	Dentin
LR2D	II	11.0- 90	12.0	Gutta percha
LR3M	II	14.0- 80	15.0-80	Gutta percha (overfill)
LR3D	III	13.0- 80	14.0	Dentin
LR4M	IV	16.0- 80	17.0	Dentin - Gutta percha
LR4D	IV	16.0- 80	17.0	Dentin - Gutta percha
UR2M	I	12.5- 80	13.5	Control (P)
UR2D	II	12.0- 80	13.0-80	Gutta percha (overfill)
UR3M	IV	12.0- 80	13.0	Dentin - Gutta percha
UR3D	III	12.0- 80	13.0	Dentin

NOTE:

Only maxillary canals contained dye solution.

TABLE V

RADIOGRAPHIC AND DYE PENETRATION RESULTS - Dog A

Premolar Canal	Group	Radiographic Changes	Dye Penetration
L1	III	No periapical changes	Partial penetration into dentin chip layer
L2M	I P	2x2 periapical radiolucency	Dye not visible
L2D	III	No periapical changes	Partial penetration into dentin chip layer
L3M	II	Excess sealer - normal periapex	No penetration
L3D	II	Excess sealer - normal periapex	No penetration
L4M	IV	4x5 diffuse periapical radiolucency	No penetration
L4D	IV	4x5 diffuse periapical radiolucency	No penetration
R1	I NP	No periapical changes	Dye not visible
R2M	III	No periapical changes-furcation defect	Partial penetration into dentin chip layer
R2D	III	No periapical changes-furcation defect	Partial penetration into dentin chip layer
R3M	II	Excess sealer - normal periapex	No penetration
R3D	II	Excess sealer - normal periapex	No penetration
R4M	IV	4x4 radiolucency - apical resorption	No penetration
R4D	IV	5x3 radiolucency - apical resorption	No penetration

TABLE VI

RADIOGRAPHIC AND DYE PENETRATION RESULTS - Dog B

Premolar Canal	Group	Radiographic Changes	Dye Penetration
L1	III	Normal periapex - fractured amalgam and tooth - contaminated	Dye not visible
L2M	I NP	Widened PDL at apex	Dye visible to apex
L2D	III	No periapical changes	Dye not visible
L3M	II	Normal periapex - crown fracture into furcation	No penetration
L3D	II	Excess sealer - normal periapex - lost amalgam	No penetration
L4M	IV	Widened PDL at apex	No penetration
L4D	IV	No periapical changes	No penetration
R1	III	No periapical changes-contaminated	Partial penetration into dentin chip layer
R2M	I P	2x3 periapical radiolucency - fractured amalgam	Dye not visible
R2D	III	No periapical changes	Partial penetration into dentin chip layer
R3M	II	Excess sealer - fractured amalgam and tooth-normal periapex	No penetration
R3D	II	Excess sealer - normal periapex	No penetration
R4M	IV	No periapical changes-lost amalgam	No penetration
R4D	IV	1x2 periapical radiolucency	No penetration

TABLE VII

RADIOGRAPHIC AND DYE PENETRATION RESULTS - Dog C

Premolar Canal	Group	Radiographic Changes	Dye Penetration
L1	I NP	No periapical changes	Dye visible in apical one-third
L2M	III	No periapical changes	Partial penetration into dentin chip layer
L2D	III	No radiographic changes	Dye not visible
L3M	II	Widened PDL at apex	No penetration
L3D	II	Excess sealer - normal periapex	No penetration
L4M	IV	No periapical changes	No penetration
L4D	IV	No periapical changes	No penetration
R1	I P	No periapical changes	Dye not visible
R2M	III	No periapical changes	Partial penetration into dentin chip layer
R2D	III	No periapical changes	Partial penetration into dentin chip layer
R3M	II	Excess sealer - normal periapex	No penetration
R3D	II	Excess sealer - normal periapex	No penetration
R4M	IV	No periapical changes	No penetration
R4D	IV	No periapical changes	No penetration

TABLE VIII

RADIOGRAPHIC AND DYE PENETRATION RESULTS - Dog D (Mandibular)

Premolar Canal	Group	Radiographic Changes	Dye Penetration
L1	III	No periapical changes	Dye not placed in canal
L2M	I P	No periapical changes	Dye not placed in canal
L2D	I NP	No periapical changes	Dye not placed in canal
L3M	III	2x3 periapical radiolucency	Dye not placed in canal
L3D	II	Excess sealer - diffuse radiolucency	Dye not placed in canal
L4M	IV	3x5 periapical radiolucency - root resorption	Dye not placed in canal
L4D	IV	3x5 periapical radiolucency - root resorption	Dye not placed in canal
R1	II	Excess gutta percha-sealer	Dye not placed in canal
R2M	III	No periapical changes	Dye not placed in canal
R2D	II	No periapical changes	Dye not placed in canal
R3M	II	Excess gutta percha-sealer - no periapical changes	Dye not placed in canal
R3D	III	3x3 periapical radiolucency	Dye not placed in canal
R4M	IV	4x5 periapical radiolucency - severe root resorption	Dye not placed in canal
R4D	IV	4x5 periapical radiolucency - severe root resorption	Dye not placed in canal

TABLE IX

RADIOGRAPHIC AND DYE PENETRATION RESULTS - Dog D (Maxillary)

Premolar Canal	Group	Radiographic Changes	Dye Penetration
L2M	II	Excess sealer -2x3 periapical radiolucency	No penetration
L2D	III	No periapical changes	Partial penetration into dentin chip layer
L3M	IV	3x2 periapical radiolucency	No penetration
L3D	I NP	No periapical changes	Dye not visible
R2M	I P	Diffuse radiolucency	Dye not visible
R2D	II	Excess gutta percha - diffuse radiolucency	No penetration
R3M	IV	External resorption of apex	No penetration
R3D	III	No periapical changes	Partial penetration into dentin chip layer

TABLE X

HISTOLOGIC RESULTS - Dog A

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
L1	III	None present	Yes	Few necrotic chips of cementum at apex - no visible evidence of resorptive defect - no inflammatory infiltrate.
L2M	I P	Large	No	Large actively resorpting defect with severe chronic inflammatory cell infiltrate.
L2D	III	Small	No	Mild chronic inflammation - granulation tissue in contact with dentin chips.
L3M	II	Medium	No	Excess sealer in PDL - Necrosis of tissue adjacent to sealer - moderate chronic inflammatory cell infiltrate.
L3D	II	Medium	No	Active bone resorption and slight cementum resorption - granulation tissue surrounding liquifaction necrosis - moderate chronic inflammation.
L4M	IV	Large	No	Severe resorption of cementum and bone-breakdown of PDL - moderate chronic inflammation.
L4D	IV	Very Large	No	Large defect with active resorption of bone and cementum - necrotic cementum at center of granuloma

TABLE XI

HISTOLOGIC RESULTS - Dog A

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
R1	I NP	None present	No	Granulation tissue in small resorptive defect in bone with mild chronic inflammation present in accessory canals.
R2M	III	3mm below apex	Yes	Well organized PDL with formation of osteoid adjacent to dentin chips - Granuloma within bony defect (perforation).
R2D	III	None present	Yes	Mild chronic inflammation within widened PDL - granulation tissue proliferating into canal.
R3M	II	Small	No	Lequifaction necrosis adjacent to extruded sealer with slight amount of cementum resorption - moderate chronic cell infiltrate.
R3D	II	Medium	No	Sealer and fragments of cementum in connective tissue surrounded by liquifaction necrosis - moderate chronic cell infiltrate.
R4M	IV	Large	No	Large defect with active resorption of bone and cementum - granulation tissue in contact with dentin chips - vital accessory canals.
R4D	IV	Large	No	Large resorptive defect of bone and cementum - granulation tissue in contact with dentin chips - moderate chronic inflammation - some accessory canals inflamed.

TABLE XII

HISTOLOGIC RESULTS - Dog B

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
L1	III	None present	Yes	Evidence of resorptive defect - granulation tissue within canal - vital accessory canals.
L2M	I NP	None present	No	Granulation in area of bone resorption - cementoid deposited in vital accessory canals - mild, scattered chronic inflammatory cells in PDL.
L2D	III	None present	Yes	Well organized connective tissue in canal - vital accessory canals - no inflammation present.
L3M	III	None present	Yes	Evidence of cementum resorption - dentin chips into PDL with osteoid formation - no inflammation present.
L3D	II	Medium	No	Area of liquifaction necrosis around sealer connective tissue fibers surrounding this material - vital accessory canals.
L4M	IV	Small	No	Evidence of resorptive defect present - granulation tissue in contact with dentin chips - mild chronic inflammatory infiltrate.
L4D	IV	Small	No	Some active bone and cementum resorption - vital accessory canals granulation tissue in contact with dentin chips.

TABLE XIII

HISTOLOGIC RESULTS - Dog B

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
R1	III	None present	Yes	Dentin chips extending to apex - excellent organization of PDL and osteoid formation.
R2M	I P	Large	No	Abscess formation with extensive resorption of bone, cementum, and dentin.
R2D	III	Very Small	No	Evidence of bone resorption - granulation tissue in contact with dentin chips.
R3M	II	Small	No	Moderate chronic inflammation around necrotic zone - cementoid formation in accessory canals.
R3D	II	Large	No	Large defect in bone - extruded sealer, necrotic tissue, chronic inflammatory cells in fibrous connective tissue capsule.
R4M	IV	None present	Yes	Evidence of large resorptive defect - osteocementum formation within main canal and accessory canals - vital accessory canals.
R4D	IV	Large	No	Chronic inflammatory cells in contact with dentin chips - repair of apical cementum - vital accessory canals.

TABLE XIV

HISTOLOGIC RESULTS - Dog C

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
L1	I NP	None present	No	Complete lamina dura with intact PDL - no evidence of resorptive defect.
L2M	III	None present	Yes	Repair of perforation with osteocementum within canal - vital accessory canals - not complete seal.
L2D	III	Large	No	Packed necrotic cells adjacent to dentin chips - moderate chronic inflammatory cell infiltrate in granulation tissue and into accessory canals.
L3M	II	Medium	No	Slight cementum resorption - extruded sealer forming center of granuloma - vital accessory canals.
L3D	II	Large	No	Definite encapsulation of extruded sealer - moderate chronic inflammatory cell infiltrate - vital accessory canals.
L4M	IV	None present	Yes	Connective tissue proliferation into canal with deposition of osteoid adjacent to dentin chips - vital accessory canals.
L4D	IV	None present	Yes	Evidence of resorption repair of cementum - apparent closure of apical foramen.

TABLE XV

HISTOLOGIC RESULTS - Dog C

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
R1	I P	Very Small	No	Evidence of resorptive defect with granulation tissue in widened PDL - very mild chronic inflammatory response.
R2M	III	None present	Yes	Evidence of bone resorption - repair with osteocementum adjacent to dentin chips - no inflammation - vital accessory canals.
R2D	III	Medium	No	Packed chronic inflammatory cells in contact with dentin chips - granulation tissue in PDL space - repair of cementum defect - vital accessory canals.
R3M	II	Small	No	Moderate chronic inflammatory infiltrate surrounding connective tissue capsule - extruded sealer and necrotic material still present - cementum resorption.
R3D	II	Small	No	Area of liquifaction necrosis around extruded sealer - vital accessory canals - evidence of cementum resorption.
R4M	IV	None present	Yes	Granulation tissue in resorbed area of bone-mild, isolated areas of chronic cells - dentin to PDL with osteoid formation.
R4D	IV	None present	Yes	Evidence of bone and cementum resorption - granulation tissue with few, scattered chronic cells - vital accessory canals.

TABLE XVI

HISTOLOGIC RESULTS - Dog D (Mandibular)

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
L1	III	None present	Yes	Excellent organization of PDL - osteocementum bridge is complete in all sections - no inflammation present.
L2M	I P	None present	Yes	Connective tissue proliferation into canal with circumferential deposition of osteocementum.
L2D	I NP	None present	No	Normal PDL with vital accessory canals.
L3M	III	Large	No	Large resorptive defect - chronic inflammation within PDL - breakdown of accessory canals.
L3D	II	Large	No	Severe chronic inflammatory cell infiltrate extending along PDL - necrotic material at center of granuloma.
L4M	IV	Large	No	Large resorptive defect with extensive inflammatory infiltrate - defect extending coronally in PDL.
L4D	IV	Large	No	Chronic inflammatory infiltrate into PDL - granuloma tissue within defect and in contact with dentin chips - spicules of bone in granulation tissue.

TABLE XVII

HISTOLOGIC RESULTS - Dog D (Mandibular)

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
R1	II	Very Large	No	Extensive inflammatory response around extruded sealer - very little connective tissue visible - no cementum resorption.
R2M	III	No reading	No reading	Response of this canal overshadowed by DLR1 and DLR2D.
R2D	II	Very Large	No	Severe chronic inflammatory cell infiltrate extending into marrow spaces - no resorption of cementum.
R3M	II	Very Large	No	Severe bone resorption - necrotic cells, liquifaction necrosis and necrotic bone within mass of chronic inflammation.
R3D	III	Large	No	Center zone of tightly packed cells with connective tissue capsule - chronic inflammation present in accessory canals.
R4M	IV	Very Large	No	Severe resorption of all supporting structures - massive chronic inflammatory cell infiltrate.
R4D	IV	Very Large	No	Extensive defect with active resorption of cementum, dentin, and bone - involved 1/3 of root - inflammatory cell infiltrate extending into marrow spaces.

TABLE XVIII

HISTOLOGIC RESULTS - Dog D (Maxillary)

Premolar Canal	Group	Granuloma	Osteocementum Formation	Comments
L2M	II	Very Large	No	Severe bone resorption - severe inflammation no attempt to wall off necrotic zone.
L2D	III	Medium	No	Granulation tissue in bony defect - mild chronic inflammatory infiltrate extending along PDL.
L3M	IV	Medium	No	Accessory canals contained chronic cells - no organization of PDL - evidence of cementum and bone resorption.
L3D	I NP	Large	No	Severe inflammatory response with extensive resorption - total disruption of PDL.
R2M	I P	Large	No	Pulpal debris at apex with connective tissue capsule - severe chronic cell response.
R2D	II	Very Large	No	Severe inflammatory response to gutta percha sealer (3mm overfill) - granuloma into maxillary sinus - no cementum resorption.
R3M	IV	Very Large	No	Total breakdown of supporting structures with proliferation of tissue into maxillary sinus - severe cementum resorption.
R3D	III	Large	No	Granulation tissue below dentin chips - severe chronic cell infiltrate into marrow spaces.

Figure 1: Preliminary specimen. Note penetration of methylene blue into dentin tubules and partial penetration of dye into dentin chip layer (arrows). (Mounted paraffin block. Magnification, X3)

Figure 2: Dye present in reservoir above gutta percha fill (arrow). No dye present in dentin tubules. (Mounted paraffin block, three-month specimen. Magnification, X3)

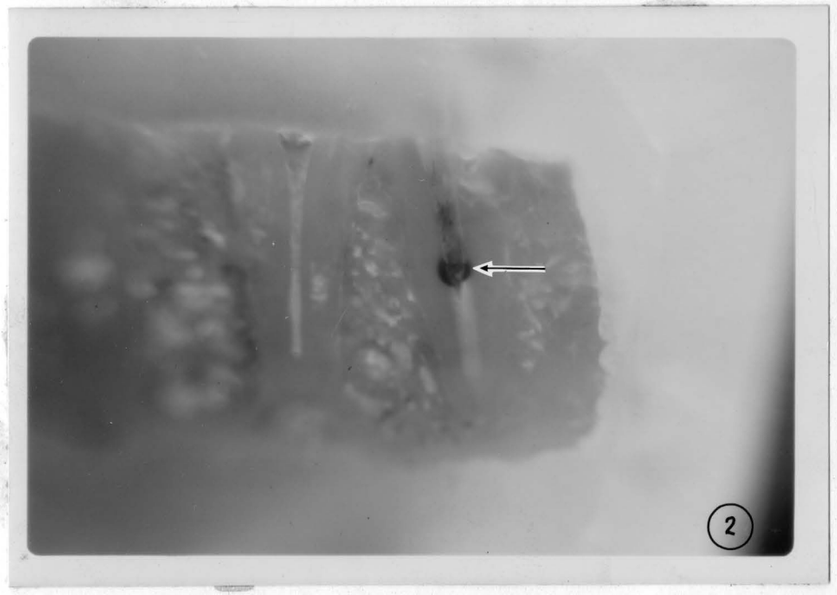


Figure 3: Note partial penetration of dye into dentin chip layer (arrow). (Mounted paraffin block, one-month specimen. Magnification, X3)

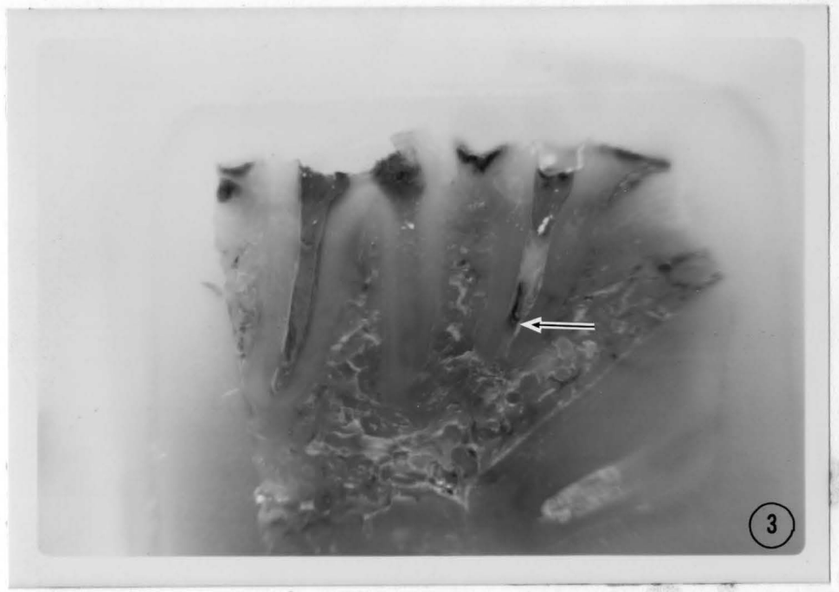


Figure 4: Retention of dye in apical one-third of non-perforated control canal. (Mounted paraffin block, four-month specimen. Magnification, X3)

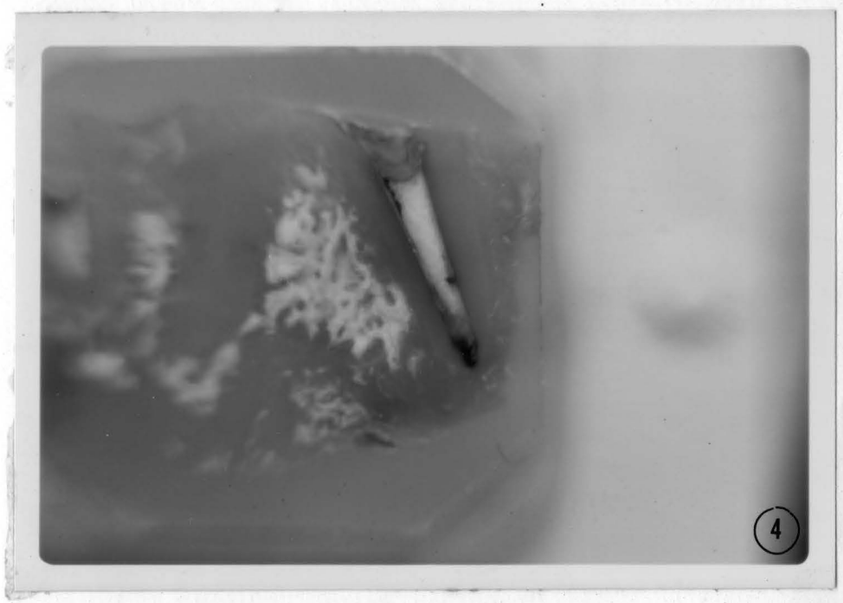


Figure 5: A Group I, nonperforated specimen. Note continuous periodontal ligament (PDL) and continuous lamina dura (LD). (Three-month specimen, hematoxylin and eosin stain. Magnification, X90)

Figure 6: A Group I, perforated specimen with apical resorption (arrows) and small granuloma. Loss of lamina dura. (Two-month specimen, hematoxylin and eosin stain. Magnification, X90)

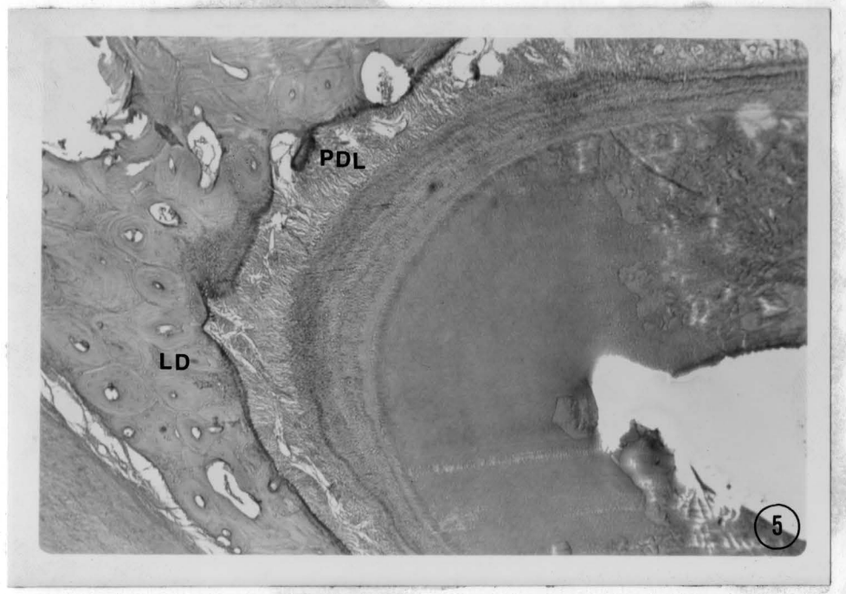
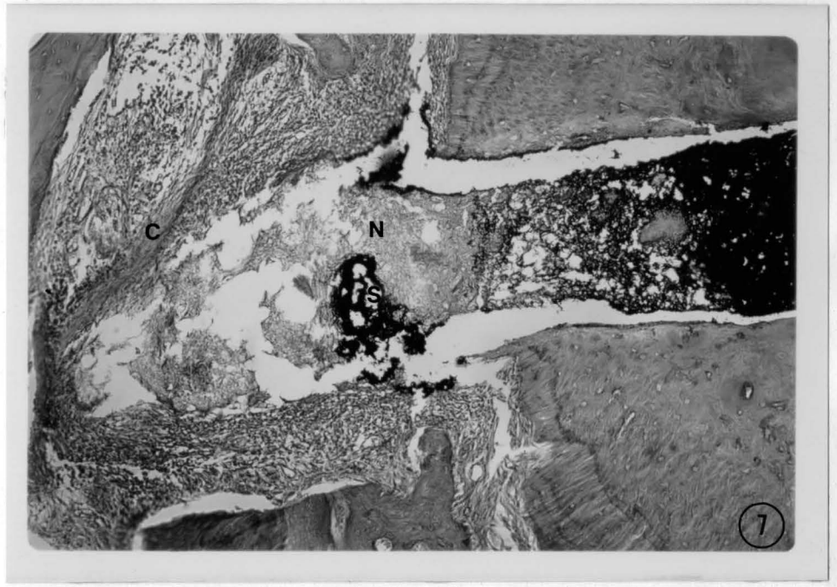


Figure 7: Extruded sealer (S) and necrotic tissue (N) surrounded by fibrous connective tissue capsule (C). (Four-month specimen, hematoxylin and eosin stain. Magnification, X90)

Figure 8: Enlargement of the fibrous connective tissue capsule (C) surrounding sealer (S). Moderate chronic inflammatory infiltrate present. Active bone resorption (arrow). (Four-month specimen, hematoxylin and eosin stain. Magnification, X405)



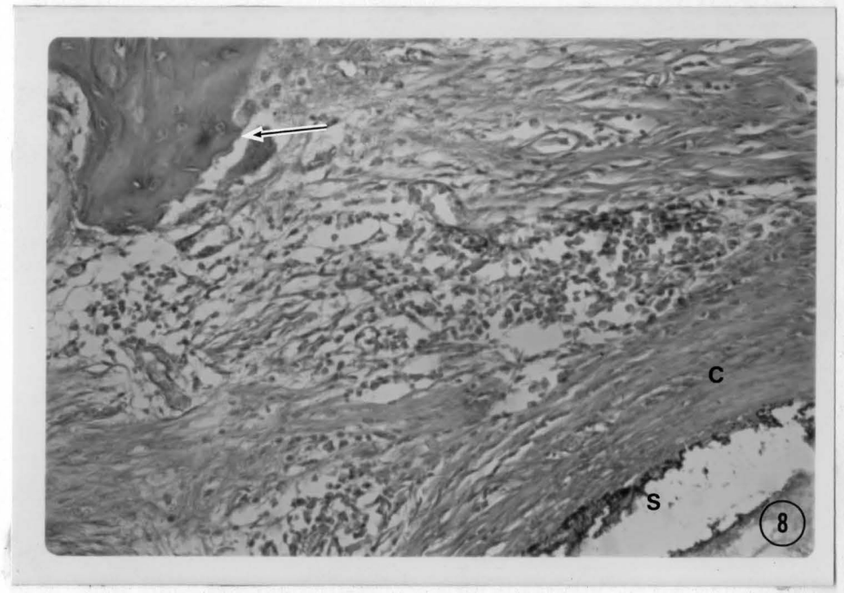
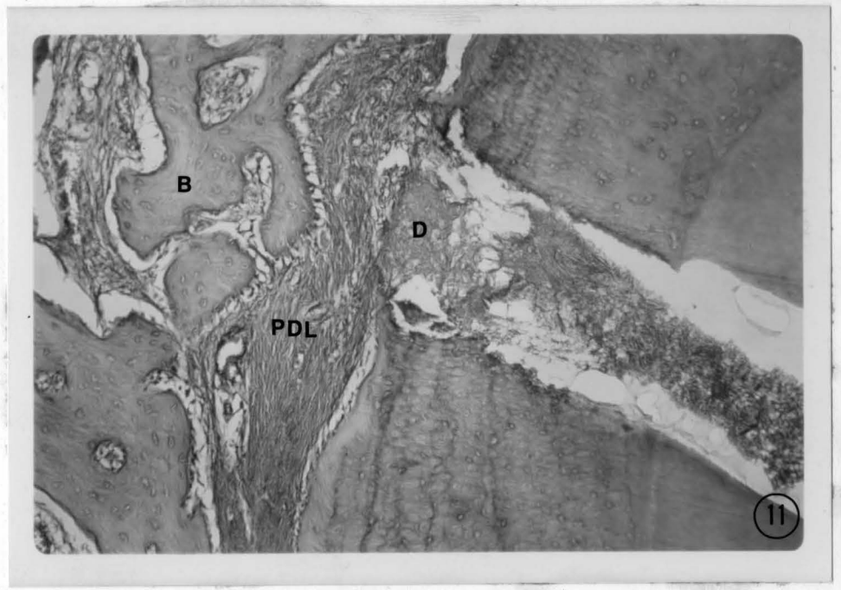


Figure 9: Connective tissue into unfilled portion of canal in contact with dentin chips (D). Granulation tissue (G) in area of bone loss. (One-month specimen, hematoxylin and eosin stain. Magnification, X90)

Figure 10: Note organization of connective tissue in contact with dentin chips (D). Cementoid present (arrows). (One-month specimen, hematoxylin and eosin stain. Magnification, X405)

Figure 11: A well organized periodontal ligament (PDL) between packed dentin chips (D) and bone (B). (One-month specimen, hematoxylin and eosin stain. Magnification, X90)

Figure 12: Note organization of connective tissue fibers between bone (B) and dentin chips (D). Periodontal ligament-like structure is present. (Hematoxylin and eosin stain. Magnification, X405)



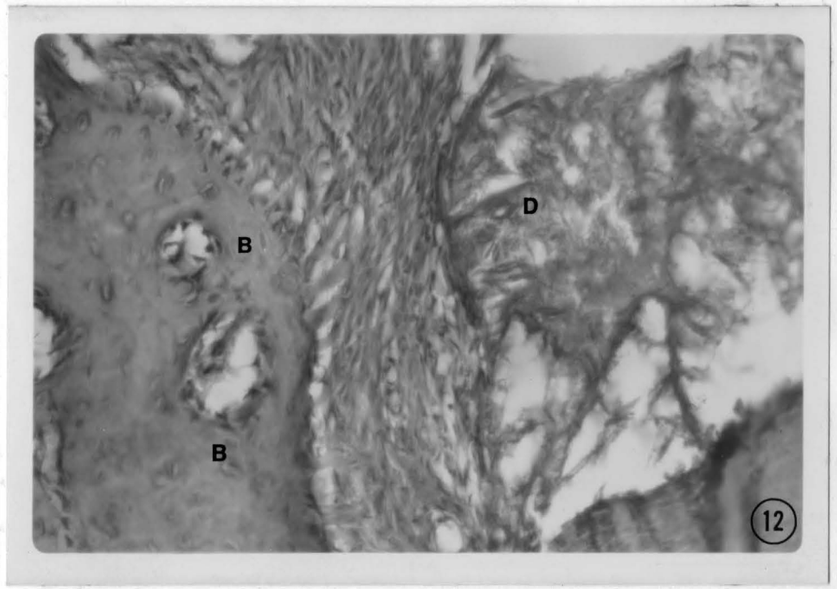


Figure 13: Initial formation of osteocementum (O) adjacent to dentin chips (D). (Two-month specimen, hematoxylin and eosin stain. Magnification, X90)

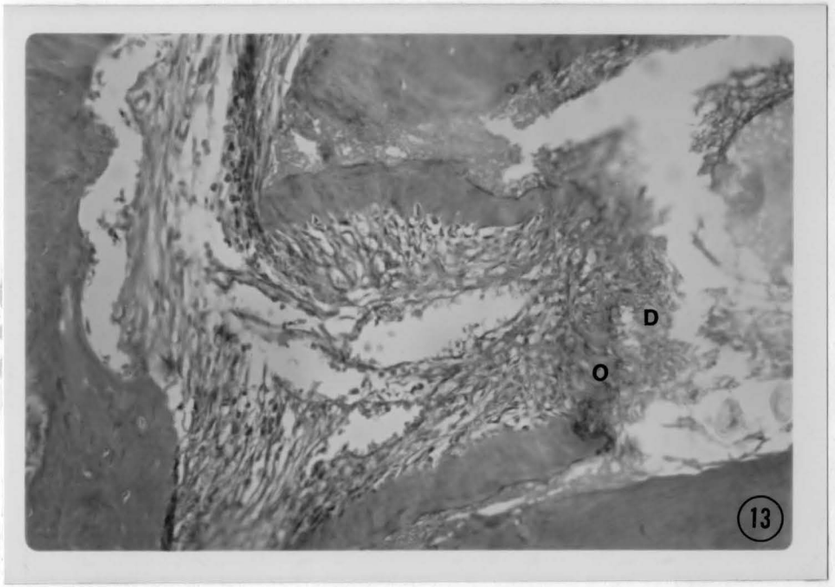


Figure 14: Cementoblast-like cells (arrows) adjacent to dentin chips (D). Note vascularity of connective tissue. (Two-month specimen, hematoxylin and eosin stain. Magnification, X405)

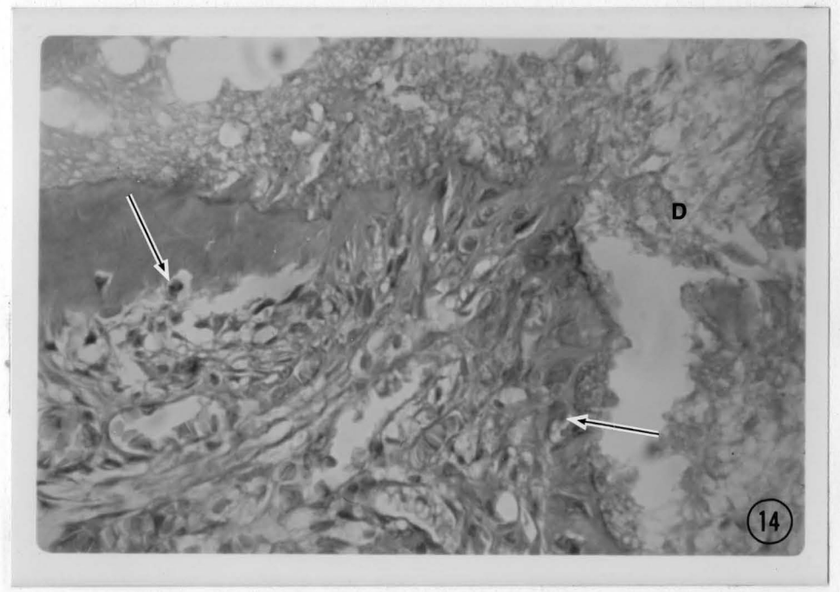
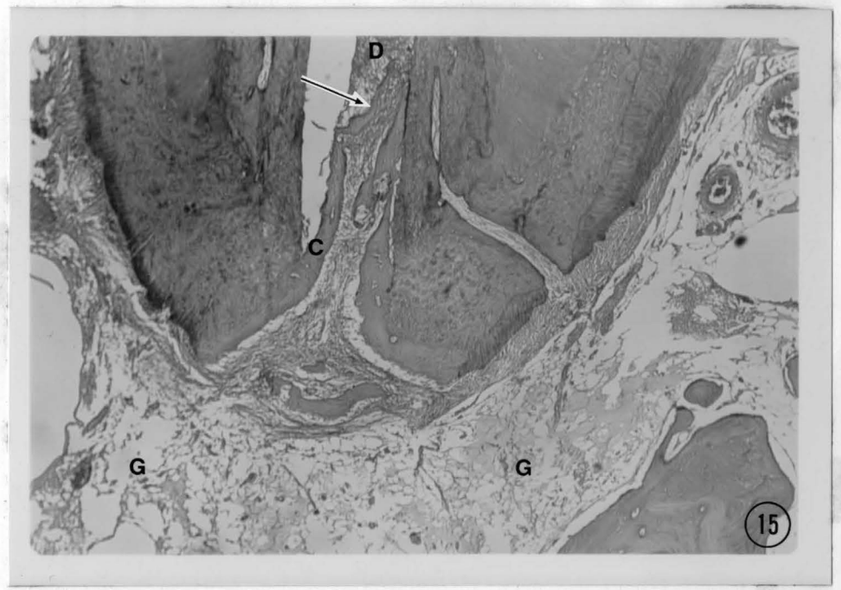


Figure 15: Granulation tissue (G) in large resorptive defect. Cementoid formation circumferentially within canal (C). Osteocementum (arrow) formation adjacent to dentin chips (D). (Three-month specimen, hematoxylin and eosin stain. Magnification, X90)

Figure 16: Osteocementum (O) formation adjacent to packed dentin chips (D). (Four-month specimen, hematoxylin and eosin stain. Magnification, X90)



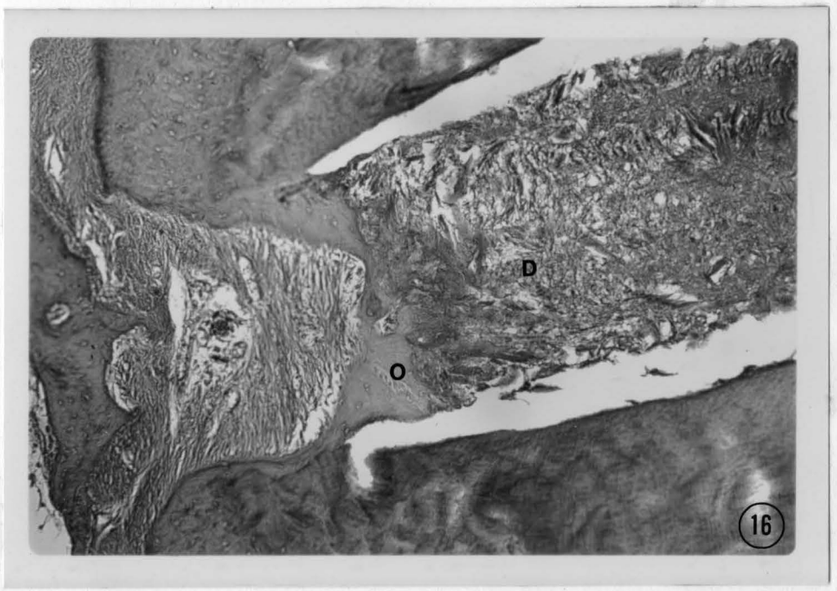
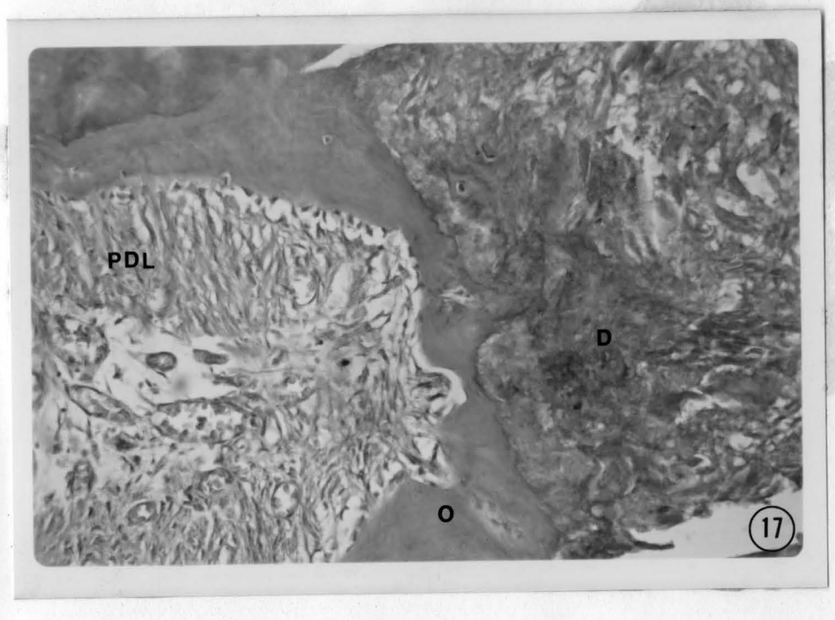


Figure 17: Note organization of the connective tissue into a periodontal ligament-like structure (PDL). Dentin chips (D) with osteocementum (O). (Four-month specimen, hematoxylin and eosin stain. Magnification, X180)

Figure 18: Cellular inclusions within osteocementum (O). Collagen fibers appear to be similar to Sharpey's fibers (S). Dentin Chips (D). (Four-month specimen, hematoxylin and eosin stain. Magnification, X405)



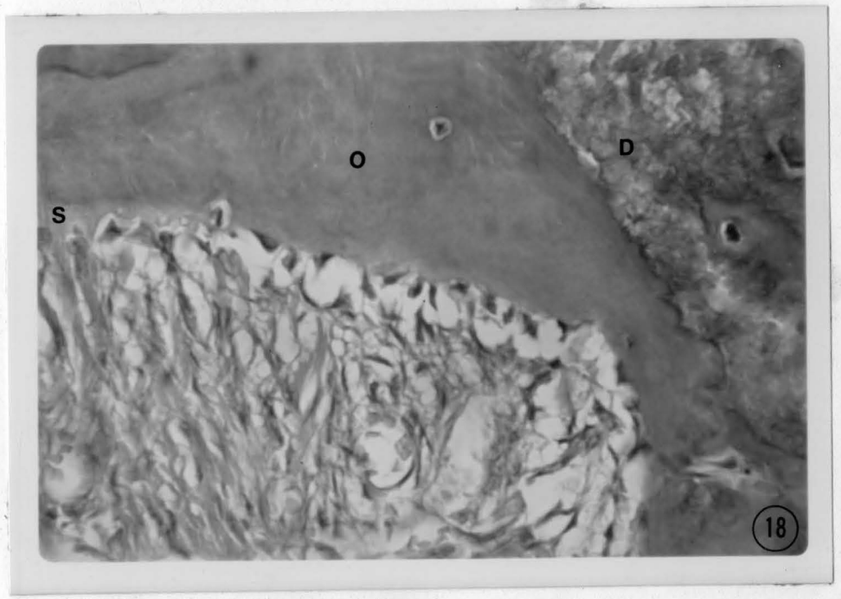


Figure 19: Overinstrumented canal. Granulation tissue in contact with packed dentin chips (D). Organization of collagen fibers into a periodontal ligament-like structure (arrows). Granuloma (G) within bony defect. (Hematoxylin and eosin stain. Magnification, X36)

APPROVAL SHEET

This thesis, submitted by Robert J. Clayton, has been read and approved by three members of the faculty of the Department of Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 18, 1973
Date

Hal D. McReynolds, Ph.D.

Hal D. McReynolds
Signature of Advisor